Title	Testing aerobic bacterial endospores as indicators for Beliche Water Treatment Plant ozone disinfection performance evaluation
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The public health risks which may arise from the occurrence of pathogenic microorganisms in drinking water supplies are such that its control is of paramount importance. Contaminated water can be a source of major disease outbreaks, particularly for immunologically compromised individuals. So, multiple barriers to pathogen transit from abstraction to the consumers' tap must be used to ensure the microbiological safety of the distributed water. Of these barriers, the disinfection with ozone is one of the most effective.

However, some pathogenic forms (*e.g., Cryptosporidium parvum* oocysts) are remarkably resistant to chemical oxidation and, therefore, their inactivation during disinfection needs to be crucially controlled. Unfortunately such task can not be fully achievable by the currently used methods, as these rely on the monitoring of inactivation rates of indicator microorganisms (heterotrophic plate counts (HPC), total coliform bacteria, thermotolerant coliforms, *Escherichia coli* and *Clostridium perfringens*) which are significantly less resistant to ozone than many waterborn pathogens (*e.g., C. parvum* oocysts, enteroviruses). Accordingly, the former are not fully appropriate as surrogate organisms, owing to their higher sensitivity to ozone. Hence, alternative indicators have been suggested, including bacterial phages and endospores. The latter being particularly suitable as surrogates, since their resistance to chemical oxidants is higher (or at least comparable) to that of *C. parvum* oocysts, one of the most resistant waterborn pathogens. Due to its resistance to disinfection and associated health hazards, elimination of *C. parvum* form drinking water is presently one of the biggest challenges in the production of water for human consumption, particularly that of surface origin.

The endospores of aerobic bacteria (*e.g., Bacillus* spp.) appear as more suitable indicators for assessing disinfection efficiency than their anaerobic counterparts (*e.g., Clostridium* spp. endospores). In addition to not conveying human health risks, they occur in surface water bodies at numbers which by far exceed those of the anaerobic species and are more amenable for counting by using routine microbiological methods.

Hence, this work aimed to test the feasibility of using aerobic bacteria endospores as indicators of ozone disinfection efficiency in Beliche water treatment plant (DWTP) and, in addition, to obtain data preliminary to a further implementation of the method.

Beliche DWTP is located in Algarve (Portugal) and receives water from the Odeleite/Beliche dam. The plant has ozonation as the first treatment step, which is followed by conventional coagulation / flocculation / clarification, rapid sand filtration and final disinfection with chlorine.

In this study, endospore and HPC numbers were determined for both raw and ozonated water, over a period of três months. In addition, during the same period endospore inactivation rates were determined, as influenced by temperature, turbidity and pH. Ozone was applied at a close to constant concentration and contact time (CT ~ 3.4 $mg \cdot L^{-1} \cdot min$).
Contrarily to HPC, which were occasionally detected in the ozonated water at very low numbers, endospores were invariably found in both raw and ozonated water at numbers allowing for their easy quantification in relatively small water samples (≤ 50 ml). Observed endospore inactivation rates ranged from 0,5 log to 1,6 log. Under the observed raw water turbidity (2,0 NTU to 0,9 NTU) and pH (7,6 to 7,2) regimes, effects of these parameters on endospore inactivation were not apparent. However, endospore inactivation rates were clearly influenced by temperature, as they decreased steadily from 1,6 log to 0,5 log, as the water temperature declined gradually from 18.7ºC to 12.9ºC over the testing period.
The suitability of aerobic endospores as surrogates for the evaluation of Beliche DWTP ozone disinfection performance was demonstrated. In addition, the results obtained so far suggest that temperature has a much stronger impact on aerobic endospore inactivation by ozone than turbidity or pH, at least within the tested range of these parameters. Such findings, which are in accordance with results obtained from laboratory scale experiments by other authors, are important for implementing the use of aerobic endospores inactivation rates to evaluate Beliche DWTP ozonation performance as a safety barrier.

References

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Topic: Water/wastewater treatment and disinfection